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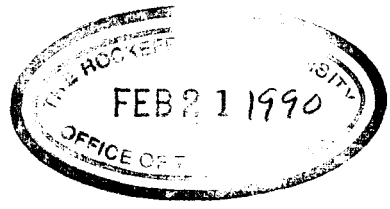

Dr. Joshua Lederberg
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1230 York Avenue
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February 15, 1990

Dear Dr. Lederberg,

It was good to hear from you regarding chromosome rearrangements and their selection or induction.

Our thesis (without compelling data) is that the ability to form rearrangements, especially duplications, provides significant selective advantage to bacteria. This advantage could be due to increased dosage of a single gene, to synergistic effects of amplifying several genes or to novel sequences (perhaps operon fusions or hybrid genes) created at the duplication junction point. Since duplications are reversible, a duplication-bearing population that expanded during a period of stress would not be committed to its new genotype. Since spontaneous frequency of duplications is generally high ($1/30 - 1/10^4$) and they do not have highly deleterious side effects, we suggest that duplications might serve as a valuable means of adaptation to stress; in effect, this would be a gene regulation mechanism whose specificity is provided by natural selection. Since duplications are lost with high frequency (usually more than one percent per generation), the advantage provided would have to be quite strong if a particular duplication were to become a substantial fraction of a population.

We've recently studied a duplication that enhances growth on several carbon sources when the level of these compounds is growth-limiting. It appears that there is strong synergistic effect of multiple genes in providing this growth advantage. At least the permease and the crp gene seem to be required within the duplicated segment. The selective advantage can be strong; at a sufficiently low concentration of carbon source the duplication grows while

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haploids fail to grow. We are still working on the basis of the observation.

You asked whether the duplication is induced or selected. We think that the duplication is selected since we can detect it in the population at about $1/10^4$ cells by means that involve no selection. The increase in frequency of the duplication in chemostats has been assumed to be due to more rapid growth, but the possibility of more rapid occurance of duplications during selection has not been eliminated. The duplication mentioned above appears to affect Mu transposition in some way, implying that it could cause some alteration in DNA metabolism. This duplication (for Salmonella) appears to be analogous to variants in E. coli described by Shapiro (J. Bacteriol. 171:5975). We have shown that his strains carry duplications that have different endpoints but overlap substatially the duplications we have studied in Salmonella.

Another area of interest that is indirectly related to this involves repeated sequences. A corrollary of the thesis that duplications are important is the prediction that selection will act to position repeated sequences so as to favor the occurance of duplications that are selectively useful. We suspect that the IS200 element, specific for Salmonella may be functioning in this way.

A postdoc here (David Thaler) is currently looking for evidence that mutation, and perhaps low stringency recombination might occur preferentially in a subpopulation of "adventurous" cells whose frequency might increase during stress. There are a few hints supporting this but it's pretty preliminary. The recent finding (by Thaler and Radman) that the stringency of recombination (defined as requirement for sequence homology) in bacterial recombination is strongly influenced by the mismatch repair system; this opens the possibility that this stringency might be regulated. If acquisition of information is occasionally sought by bacteria, one might expect that restriction systems would be regulated as well.

Inversions and barriers to their occurance and detection are another interest here. The extremely low frequency of reporting of such mutations probably lies in the fact that phenotypes permitting detection depend on the sequence disruptions at the two join points. It is unlikely that substantial repeated sequences would be located within genes to permit these events. For duplications, we detect all

duplications that generate two copies of a particular point, regardless of where the endpoints of the duplication lie. Thus for duplications any repeated sequences that flank the assay point can serve to permit the rearrangement. Duplications are frequently reported and arise at high frequency for all points in the chromosome except the replication terminus.

When extensive sequences are deliberately placed in inverse order the chromosome, recombination results in inversion of the intervening segment for some but not all positions at which these sequences are placed. We have suggested a model for recombination that we think might account for this observation. We suggest that every recombination event involves an intermediate which has a double strand break and that these ends are subject to degradation. If degradation proceeds outside the embedded region of homology, completion of the recombination event (formation of an inversion) is impossible. Perhaps some regions of the chromosome are more prone to degradation of broken ends.

Sorry to be so long winded. I include some reprints that touch on these points.

Sincerely yours,

John Roth.

John Roth